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13. SUPPLEMENTARY NOTES

14. ABSTRACT: The overall objective of this proposal is to evaluate the therapeutic efficacy of human, bone marrow-derived mesenchymal stem cells (MSCs) in a well-characterized mouse model of inflammatory bowel disease (IBD). Shortly after my relocation from LSU Health Sciences Center (LSUHSC) to my current position at Texas Tech Health Sciences Center (TTUHSC), we encountered an unexpected problem with our mouse model of IBD. We found that the incidence of intestinal inflammation in these mice was only 30-40% compared to our 15 year historical incidence of ~85% we observed at LSUHSC. Following a year of investigations, we identified the problem and were successful in "re-establishing" the model with an incidence of >95%. Although these investigations significantly delayed the start of the studies outlined in Task 1, we were able to begin experiments to ascertain the immuno-suppressive activity of human MSCs in our new and improved mouse model of IBD. These studies are currently ongoing. In addition, we made substantial progress in developing a more immunologically-relevant *in vitro* system to assess suppressive activity and mechanisms of MSCs that more closely models the cellular and immunological interactions that are thought to occur in our mouse model of IBD *in vivo*. Furthermore, we present exciting new data demonstrating that activation of MSCs with pro-inflammatory cytokines that are known to be overproduced in mouse and human IBD, induces the dramatic up-regulation of several different immuno-suppressive mediators. Finally, we present new data that utilizes a novel, highly sensitive and quantitative method for simultaneously measuring the homing of human MSCs to several different mouse tissues during the development of chronic gut inflammation.

5. SUBJECT TERMS
inflammatory bowel disease; mesenchymal stem cells; Tregs; IL-10, TGFβ; colitis; intestinal inflammation; immunosuppression

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1. INTRODUCTION

The inflammatory bowel diseases (IBD; Crohn's disease; ulcerative colitis) are chronic inflammatory disorders of the small bowel and/or colon that affects approximately 1.5 million people in the US with a calculated *annual cost* for both medical expenses and work loss of almost $4 billion dollars. A recent study analyzing the Department of Veterans Affairs database from 1975-2006 reports that although rates of hospitalization for ulcerative colitis (UC) and Crohn's disease (CD) have begun to stabilize over the past few years, there has been a disproportionate increase in rates of hospitalizations for nonwhite vs. white US military veterans for both UC and CD. Currently, there are only a handful of medical treatments available to treat these debilitating inflammatory disorders with only a few new therapies projected to be available in the near future. Thus, there is a clear need for the development of additional therapeutic agents to treat patients with IBD. A great deal of excitement has been generated from recent studies demonstrating that adoptive transfer of syngeneic, allogeneic or xenogeneic (*human*) MSCs suppress the inflammation and tissue injury observed in animal models of autoimmune encephalomyelitis, allograft rejection, arthritis and graft vs. host disease. Because MSCs can be grown and expanded *in vitro* and exert their immuno-regulatory activity across major histocompatibility complex barriers *in vivo*, we are in the unique position to evaluate the therapeutic efficacy of *human* MSCs in our mouse model IBD. The ***overall objective*** of this proposal is to evaluate the therapeutic efficacy of *ex vivo*-generated, bone marrow-derived human MSCs in a well-characterized mouse model of *chronic* colonic inflammation. *Hypothesis:* We propose that *ex vivo*-generated MSCs suppress chronic gut inflammation by homing to the mesenteric lymph nodes (MLNs) and/or colonic lamina propria where MSC-derived TGFβ suppresses/limits the generation of colitogenic T-cells and/or induces the formation of IL-10-producing Tregs. In order to test this hypothesis we propose the following three specific aims: a) Evaluate the ability of human MSCs to suppress the ***induction*** of chronic gut inflammation; b) Determine the therapeutic efficacy of human MSCs in reversing ***preexisting*** colitis; and c) Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis.

2. KEYWORDS

inflammatory bowel disease; mesenchymal stem cells; Tregs; IL-10, TGFβ; colitis; intestinal inflammation; immuno-suppression;

3. ACCOMPLISHMENTS

Major Goals of the Project

Task 1. Evaluate the ability of human MSCs to suppress the induction of chronic gut inflammation (months 1-12).
Task 2. Determine the therapeutic efficacy of human MSCs in attenuating preexisting colitis (months 12-24).
Task 3. Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis (months 24-36).

Accomplishments for the Current Reporting Period

Task 1. Evaluate the ability of human MSCs to suppress the induction of chronic gut inflammation

In order to assess the therapeutic efficacy of human MSCs in a mouse model of chronic gut inflammation we propose to use our well-characterized T cell transfer model of chronic colitis. We induce chronic disease by adoptive transfer of *naïve* ($CD4^+CD45RB^{high}$) T-cells obtained from healthy wild type donors into recombinase-activating gene-1 deficient ($RAG\text{-}1^{-/-}$) mice. We have more than 15 years of experience with this mouse model in which we have routinely demonstrated that ~85% of the reconstituted mice develop moderate-to-severe colonic inflammation by 6-8 weeks post T cell transfer.

Shortly after my relocation from LSU Health Sciences Center (LSUHSC) to my current position at Texas Tech Health Sciences Center (TTUHSC), we were surprised to find that only 30-40% of the RAG-$1^{-/-}$ mice housed in the TTUHSC animal facility developed chronic colitis at 8 weeks following T cell transfer compared to our historical incidence (at LSUHSC) of ~85%. We have spent the past year investigating the reason(s) for this large reduction in disease incidence. After ruling out animal vendor, housing conditions (ventilator, micro-isolator cages) and T cell preparations/administration, we determined that the reduced incidence was due to large and significant differences in the microbial composition of mice housed in the animal facilities at the 2 different institutions (Figure 1). Indeed, this situation has been described by other investigators using different mouse models of chronic disease who have changed institutions.

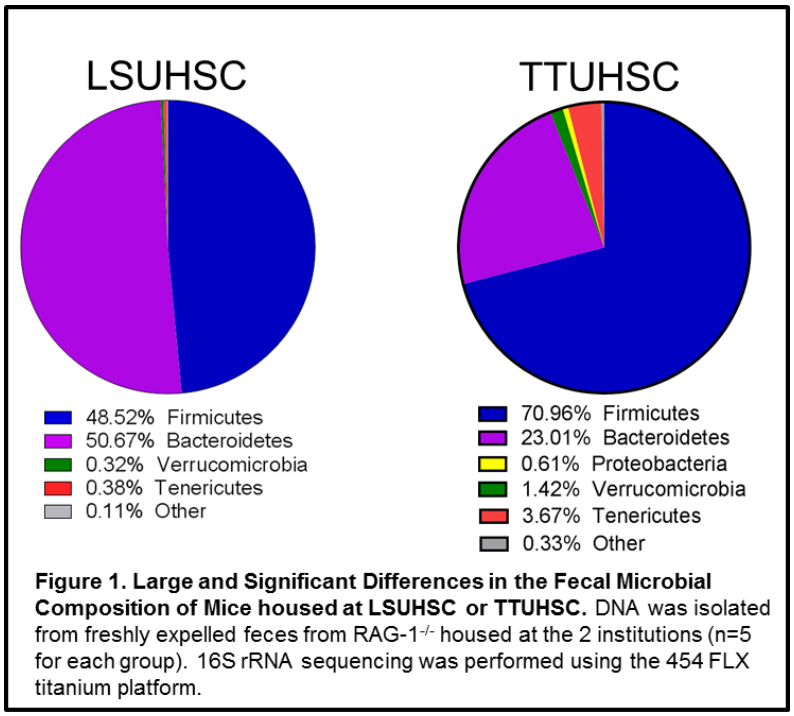

Figure 1. Large and Significant Differences in the Fecal Microbial Composition of Mice housed at LSUHSC or TTUHSC. DNA was isolated from freshly expelled feces from RAG-$1^{-/-}$ housed at the 2 institutions (n=5 for each group). 16S rRNA sequencing was performed using the 454 FLX titanium platform.

In an attempt to enhance the incidence of disease, we colonized (via gavage) RAG-$1^{-/-}$ mice housed at TTUHSC with feces obtained from colitic mice generated at LSUHSC for 1 wk prior to T cell transfer. We found that transplant of this dysbiotic microbiota induced robust colitis in >90% of these mice *following T cell transfer* (referred to as LSUHSCc→TTUSHC mice). In addition, we found that colonization of RAG-$1^{-/-}$ mice with feces obtained from colitic LSUHSCc→TTUSHC mice and then injected with naïve T cells induced moderate-to-severe colitis in virtually all (>95%) of the mice (referred to as TTUHSCc→TTUHSC mice) (Figure 2). Furthermore, we determined that colonization of healthy wild type (WT) or RAG-$1^{-/-}$ mice with colitic feces from TTUHSCc→TTUHSC mice did not induce colonic inflammation during the 8 wk observation period suggesting that: a) intestinal pathogens were not responsible for induction of disease and b) chronic gut inflammation could be produced only in mice with a dysregulated immune system (Figure 2). Although these studies delayed the start of experiments outlined in Task 1, we are very excited about the results because we now have the ability to produce robust disease in essentially all mice following colitic fecal transplant in which the microbial composition can be quantified, recorded and stored at -80°C for future use.

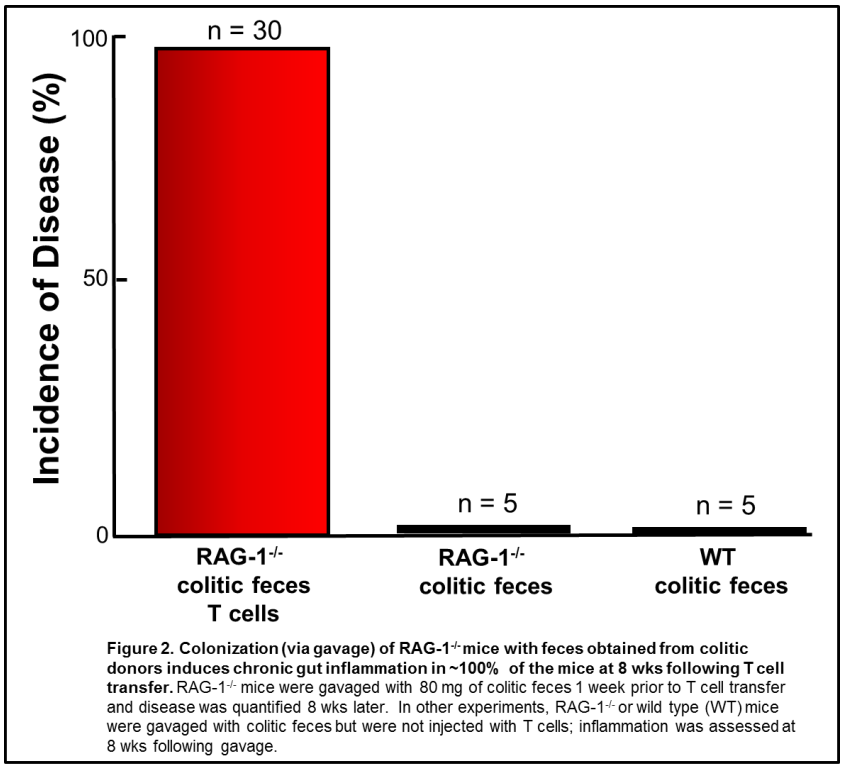

Figure 2. Colonization (via gavage) of RAG-$1^{-/-}$ mice with feces obtained from colitic donors induces chronic gut inflammation in ~100% of the mice at 8 wks following T cell transfer. RAG-$1^{-/-}$ mice were gavaged with 80 mg of colitic feces 1 week prior to T cell transfer and disease was quantified 8 wks later. In other experiments, RAG-$1^{-/-}$ or wild type (WT) mice were gavaged with colitic feces but were not injected with T cells; inflammation was assessed at 8 wks following gavage.

Preliminary data in our original application (generated at LSUHSC) demonstrated *proof of concept* that weekly injections (*i.p.*) of 5 million human MSCs for six weeks

significantly suppressed the development of chronic colitis in our mouse model of IBD. However, new clinical information has become available suggesting that the dose of MSCs we used in our original preliminary studies (166 million/kg for a 30 g mouse) are considered too large and will never be used in the clinics. Current clinical studies use either "low dose" (2 million/kg) or "high dose" (8 million/kg) human MSCs to treat different autoimmune and chronic inflammatory diseases including IBD (http://clinicaltrials.gov/show/NCT00294112). In view of this clinical reality, we have recently initiated a series of experiments to determine whether adoptive transfer of clinically-relevant doses of human MSCs suppress the development of intestinal inflammation in our new and improved mouse model of IBD. These studies are ongoing and should be completed within the next 6-8 weeks.

Task 3. Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis

To investigate the cellular and immunological interactions that occur between *human* MSCs and *mouse* immune cells in our mouse model of IBD, we have developed a xenogeneic *in vitro* system to quantify these interactions in more controlled, immunologically-relevant environment. This is an important, yet under-appreciated aspect of MSC therapeutic research in which human MSCs are used in mouse models of disease. The vast majority of published studies that treat mouse models of chronic inflammation with human MSCs have used syngeneic *in vitro* assays to model their *in vivo* studies. That is, investigators have assessed the ability of *human* MSCs to suppress activation of *human* T cells *in vitro*. We believe that it is critical to model the interactions between MSCs and immune cells in the more immunologically-relevant system using *human* MSCs and *mouse* immune cells. Therefore, as a first attempt to more closely mimic the *in vivo* situation, we quantified the immunosuppressive activity of human MSCs toward mouse T cells by incubating 100,000 mouse splenocytes with varying numbers of unstimulated human MSCs or MSCs that had been activated with IFNγ (100U/ml) + TNFα (10ng/ml) for 24 hrs prior to their 72 hr co-culture with the splenocytes. *Both of these pro-inflammatory cytokines are known to be overproduced in both mouse and human IBD*. T-cell proliferation was induced by addition of a monoclonal antibody directed towards mouse CD3. Proliferation was quantified by pulsing each well with ^{3}H-thymidine for the last 16 hrs of incubation. We found that while addition of 50,000 unstimulated MSCs significantly suppressed mouse T cell proliferation by approximately 25%, addition of 25,000, 50,000 or 100,000 cytokine-activated MSCs suppressed T cell proliferation by 58, 75 and 90%, respectively (Figure 3). These data suggest that inflammatory cytokines enhance significantly the ability of human MSCs to suppress the activation/proliferation of mouse T cells. We are currently in process of developing a novel *in vitro* system to assess the immmuno-modulatory activity of human MSCs towards mouse immune cells that are activated in an antigen-specific manner. Data obtained from

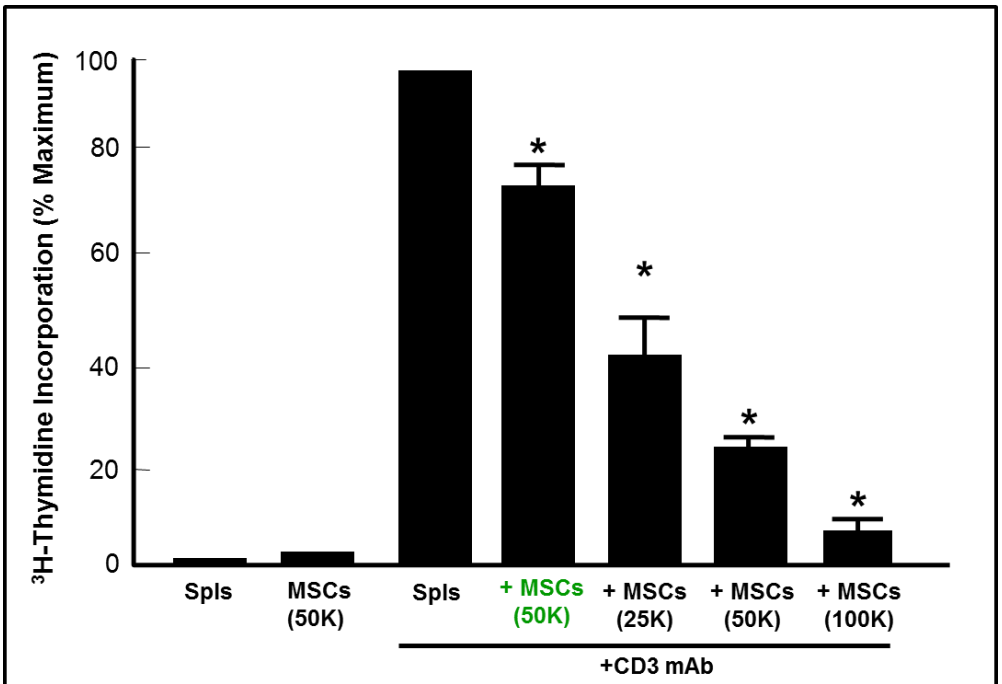

Figure 3. *Human* MSCs Suppress *Mouse* T-cell Proliferation. Mouse splenocytes (Spls; 1x10^{5}/well) were incubated alone or together with varying numbers of ***untreated MSCs*** or human MSCs that had been activated with IFNγ + TNFα for 24 hrs prior to the 72 hr co-culture with splenocytes. T-cell proliferation was induced by addition of monoclonal antibody to mouse CD3 (CD3 mAb) and proliferation was quantified by pulsing each well with ^{3}H-thymidine for the last 16 hrs of incubation. Data represent the mean ± SEM for triplicate determinations. *p<0.05 compared to Spls + CD3 mAb group.

these new, antigen-specific model systems will allow us to compare and contrast those findings generated from our current *in vitro* system that activates T cells via polyclonal activation. Our ultimate goal for this sub-aim is to develop an *in vitro* model system that recapitulates many or most of those immunological events that are responsible for the priming, polarization and expansion of colitogenic effector cells.

Our *in vitro* studies clearly demonstrate that inflammatory cytokine-induced activation of MSCs enhances significantly their suppressive activity. We next wished to ascertain which of the many candidate immunosuppressive genes are up-regulated by exposure of MSCs to the two major inflammatory mediators IFNγ and TNFα. To do this, we performed real-time (RT)-PCR using a customized, human MSC-targeted, 96 gene array kit (Bar Harbor Biotechnology, Inc.). RT-PCR data from triplicate samples of unstimulated and cytokine-stimulated MSCs were analyzed using the proprietary Global Pattern Recognition (GPR) analysis tool provided by Bar Harbor for data normalization. Data was expressed as fold increase in mRNA expression of the cytokine-stimulated vs. unstimulated cells. We found a total of 9 genes whose expression were significantly increased by >4-fold following incubation with the two cytokines ($p<0.05$). Figure 4 shows that cytokine stimulation induced the dramatic and significant up-regulation of indoleamine-2,3 dioxygenase-1 (IDO1; >100,000-fold). This is a particularly exciting observation given both the magnitude of the increase of this gene but also because IDO1 has been implicated as a major immunosuppressive gene for regulatory T cell (Treg) function. IDO1 catalyzes the degradation if tryptophan thereby depleting this important amino acid that is required for T cell proliferation. Currently, little is known about the role that IDO1 may play in MSC-mediated suppression of chronic disease. In addition to IDO1, we found that TNF alpha-induced protein-6 (TNFAIP6) mRNA expression was induced by more than 140-fold following cytokine activation (Figure 4) Again, this is very exciting as our collaborator (Dr. Darwin Prockop) has shown that TNFAIP6 plays an important role in MSC-mediated protection of cardiac post-ischemic injury in mice. Dr. Prockop has offered to provide us with purified TNGAIP6 for our future *in vitro and in vivo* studies.

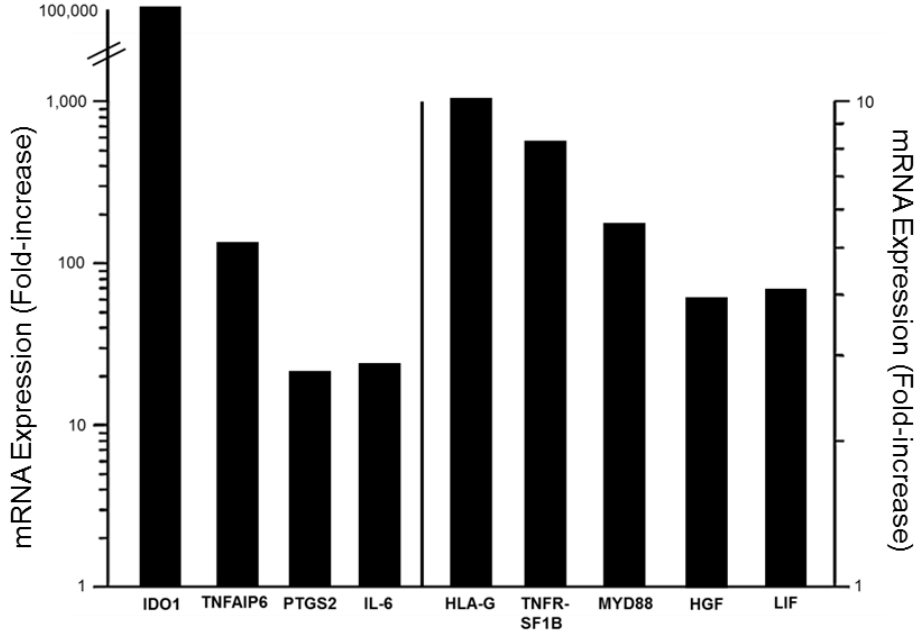

Figure 4. Cytokine-Induced Up-regulation of Immuno-Regulatory Genes in Human MSCs. Human MSCs (10^6 cells/well) were incubated in absence or presence of IFNγ (100U/ml) + TNFα (10ng/ml) for 24 hrs. Cells were washed, RNA extracted and RT-PCR was performed using a human MSC-targeted, 96 gene StellARray kit. RT-PCR data from triplicate samples of unstimulated and cytokine-stimulated MSCs were analyzed using the proprietary Global Pattern Recognition (GPR) analysis tool for data normalization. All data is reported as the mean fold-increase of cytokine-activated MSCs vs. unstimulated MSCs. All data is significant with $p<0.05$.

Our expression data have also identified two genes with known immuno-modulatory properties that were remarkably and significantly up-regulated following cytokine activation. We found that interleukin-6 (IL6) as well as prostaglandin-endoperoxide synthase-2 (PTGS2) were up-regulated by 25- and 20-fold, respectively following cytokine activation (Figure 4). Enhanced IL-6 expression is rather surprising given its well-known pro-inflammatory properties. However it is becoming increasingly apparent that IL6 is actually a pleiotropic cytokine that possesses both pro- as well as anti-inflammatory properties. Indeed, IL6 has been shown to promote, via its classical receptor-mediated signaling pathway, STAT-3-dependent regeneration of intestinal epithelial cells *in vivo.* The dramatic overexpression of MSC-derived

PTGS2 (also known as cylooxygense-2 or COX-2) also identifies an attractive immune-modifying target gene because: a) COX-2 is known to produce certain anti-inflammatory prostaglandins (e.g. PGE2 and prostacyclin) and b) selective COX-2 inhibitors for humans are readily available and can be used to interrogate the role of this MSC-derived enzyme in our model of IBD. The other 5 genes that are significantly up-regulated following cytokine activation are human leukocyte antigen-g (HLA-G; 10-fold), tumor necrosis factor receptor superfamily member 1B (TNFRSF1B; 8-fold), myeloid differentiation primary response gene 88 (MYD88; 6-fold), hepatocyte growth factor (HGF; 4-fold), and leukemia inhibitor factor (LIF; 4-fold)(Figure 4). Although all these genes have been implicated as important determinants for the immuno-regulatory properties of MSCs, few if any of these studies have been performed using *in vitro* systems that model the xenogeneic interactions that occur between human MSCs and mouse immune cells *in vivo*. In addition, no studies have been performed in animal models of chronic disease that directly assess the role of these genes using human MSCs.

One of the major questions facing investigators attempting to identify the mechanisms by which MSCs suppress chronic inflammation *in vivo* is whether MSCs need to be present within the inflamed tissue to exert their immuno-regulatory effects. While some investigators report that MSCs need not be present to exert their anti-inflammatory effects, others have shown that MSCs home to inflamed or damaged tissue where they suppress ongoing pathology. In an attempt to answer this question, we have developed a novel, highly sensitive and quantitative method to simultaneously measure the homing of human MSCs to several different mouse tissues during the development of chronic colitis. Data presented in Figure 5 show our results in which we quantified the numbers of MSCs within several different mouse tissue at 20 hrs following injection of ^{51}Cr-labeled MSCs ($5x10^6$ cells) into RAG-$1^{-/-}$ mice that had been reconstituted naïve T-cells two weeks previously. To insure that we quantified MSC-associated ^{51}Cr within the tissue interstitium and not free radioisotope or ^{51}Cr-MSCs present within the vasculature, we exsanguinated the mice and subjected the animals to exchange transfusion of the entire blood volume prior to removing and

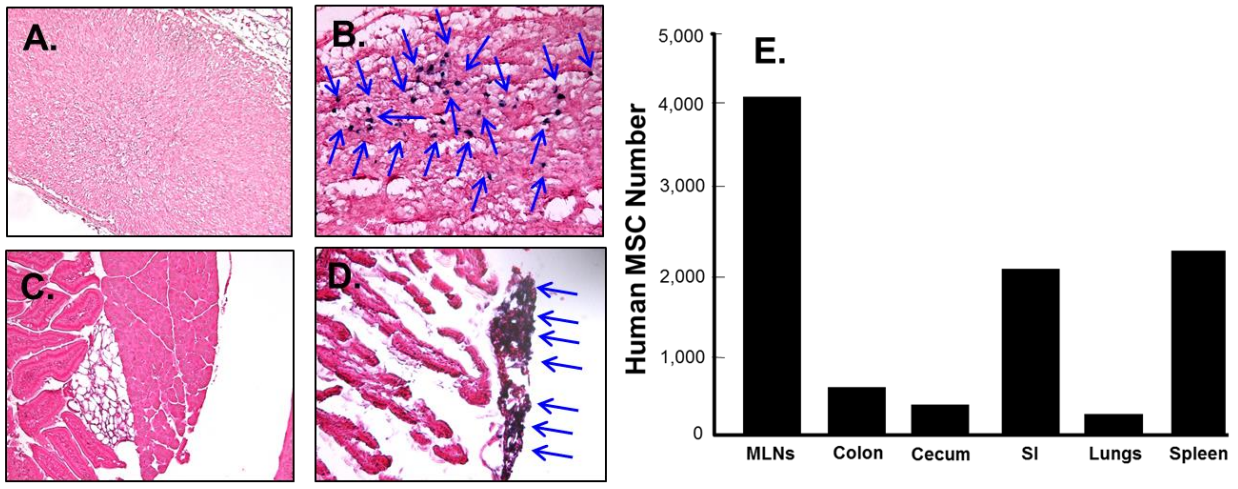

Figure 5. Presence of *human* MSCs within different mouse tissue. (A) and **(B)** represent *Negative and Positive controls,* respectively, for *in situ* hybridization of *human* Alu repeat sequences (35,36) within the MLNs at 7 days following injection of *human* MSCs (ip; $5x10^6$ cells/injection) into RAG$^{-/-}$ mice reconstituted with naïve T-cells 2 wks previously. **(C)** and **(D)** *Negative and Positive controls,* respectively, for Alu positive human cells within the small intestine of RAG$^{-/-}$ mice treated in the same manner as described in (A) and (B). 200X. **(E)** Human MSC numbers within different mouse tissue 20 hrs following injection of ^{51}Cr-labeled MSCs (ip; $5x10^6$ cells) into RAG$^{-/-}$ mice (n=3) reconstituted with naïve T-cells 2 wks previously. Radioactivity was associated with cells within tissue (and not blood) as mice were exsanguinated and subjected to exchange transfusion of the entire blood volume prior to counting tissue. Cell number was calculated base upon the specific activity of injected ^{51}Cr-labeled MSCs. ***SI***=small intestine.

counting each tissue. Cell numbers were calculated based upon the specific activity of injected ^{51}Cr-labeled MSCs. In addition, we compared our ^{51}Cr-MSC data to the more tedious immuno-histochemical method using *in situ* hybridization of *human* Alu repeat sequences (Figure 5). We found that the ^{51}Cr method agreed well with the *in situ* method however radiolabeled method is much simpler, more sensitive and more objective. It is interesting to note that MSCs home to and accumulate within the MLNs in significant numbers early in the development of chronic gut inflammation suggesting that MSCs may

interact with naïve T cells in this lymphoid tissue during the priming, polarization and/or expansion of the colitogenic effector cells.

Opportunities for Training and Professional Development

Although the primary purpose of this application was not to provide training and professional development, my postdoctoral fellow has received extensive training in the use of mouse models of IBD and flow cytometry.

Dissemination of Results

Because of the time required to "reestablish" our mouse model of IBD at our new institution, we were not able to fully implement the *in vivo* studies outlined in Task 1. Some of the preliminary data was presented in invited seminars at Georgia Reagents University (March, 2014), at Texas Tech University (December, 2014) and as a poster at the *"2014 Advances in Inflammatory Bowel Diseases" in the journal Inflammatory Bowel Diseases"* in Orlando Florida in December 2014.

Plans for the Next Funding Period

Now that we have established a new and improved mouse model of IBD, we plan to devote our full attention to Task 1 and 2. We plan to ascertain the optimal number of clinically-relevant numbers of MSCs that maximally suppresses the development of chronic colitis. We also plan to begin studies to assess the role of MLNs in MSC-mediated suppression of disease.

4. Impact

Impact on discipline

We believe that data generated from our proposed studies will impact greatly those investigators using mouse models of IBD to evaluate therapeutic efficacy of different biologics and cell-based therapies. Our new and improved model of IBD demonstrates that chronic gut inflammation can be produced on demand with a very high penetrance of disease in any animal facility. Data generated from our new studies will also impact how investigators interpret their *in vitro* data describing the immunosuppressive properties of human MSCs in mouse models of autoimmune and chronic inflammatory diseases. The vast majority of published studies using human MSCs in mouse models of disease have not examined directly the immuno-regulatory properties of *human* MSCs towards *mouse* immune cells *in vitro*. We believe that it is critical to model the interactions between MSCs and immune cells in the more immunologically-relevant system using *human* MSCs and *mouse* immune cells. Another aspect of our ongoing studies that will impact the discipline is our discovery that inflammatory cytokine-activated MSCs induce the dramatic up-regulation of certain immunosuppressive mediators that have not been interrogated in animal models of chronic inflammation. These studies may reveal new targets for drug development. Finally, we believe that our novel ^{51}Cr labeling method for quantifying MSC trafficking to multiple tissues will provide investigators with a quantitative and very sensitive method to simultaneously measure MSCs in several different mouse tissues during the onset and progression of disease. Indeed, this method may be used in conjunction with different imaging instrumentation to monitor MSC trafficking in the living animal.

Impact on other disciplines

Nothing to report

Impact on technology transfer

Nothing to report

Impact on society

Nothing to report

5. Changes/Problems

Changes in Approach
No major changes. Based upon new data from clinical studies using human MSCs in the treatment of different diseases, we will concentrate our efforts on evaluating the immunosuppressive properties of *clinically-relevant* doses of human MSCs.

Problems and Delays
Because of the time required to "reestablish" our mouse model of IBD at our new institution, we were not able to fully implement the *in vivo* studies outlined in Task 1. Fortunately, we have recently initiated the first of these studies with our new and improved model of IBD.

Changes that had a significant impact on expenditures
No major impact on expenditures

Changes in use of vertebrate animals
We have generated a new and improved mouse model of IBD.

6. Products

Publications
We published an abstract for the *"2014 Advances in Inflammatory Bowel Diseases"* in the journal **Inflammatory Bowel Diseases; Volume 20-Supplement 1; p S95.** See attached PDF.

Presentations
Some of the preliminary data was presented in invited seminars at Georgia Reagents University (March, 2014) and at Texas Tech University (December, 2014) and as a poster at the *"2014 Advances in Inflammatory Bowel Diseases" in the journal Inflammatory Bowel Diseases"* in Orlando Florida in December 2014.

7. Participants and Other Collaborators

Individuals working on project

Name: Matthew Grisham, PhD
Project Role: PI
Nearest person months: 4 months
Contribution: Dr. Grisham is involved in designing, implementing and interpreting the experiments
Funding support:

Name: Iurii Koboziev, PhD
Project Role: Postdoctoral fellow
Nearest person months: 6 months
Contribution: Dr. Koboziev performs all cell preparations, molecular and *in vitro* assays and assists with the flow cytometry studies and animal model
Funding Support:

Name: Kathryn Furr, MS
Project Role: Laboratory Scientist
Nearest person months: 5 months

Contribution: Ms. Furr is my Lab Manager and Flow Cytometry Core Manager. She performs all flow cytometry experiments; She is generates the mouse model of IBD and assists with some of the MSC cell culture experiments.
Funding Support: TTUHSC/State Account

Name: Cecily Haley, PhD.
Project Role: Postdoctoral Fellow
Nearest person months: 6 months
Contribution: Dr. Haley performs all MSC cell culture work i.e. expanding, growing and characterizing each lot of human MSCs. She is also responsible for assessing the immuno-suppressive activity of the MSCs in vitro. She is involved in many of the flow cytometry experiments and helps with the generation of the mouse model of IBD.
Funding Support:

8. Special Reporting Requirements

Nothing to report

9. Appendices

Abstract (next page) from the *"2014 Advances in Inflammatory Bowel Diseases"* in the journal **Inflammatory Bowel Diseases; Volume 20-Supplement 1; p S95.**

immunofluorescence analysis. VEGFR-3mRNA expression in colon tissue was examined by quantitative real-time RT-PCR.

RESULTS: The VEGF-C-treated mice by recombinant adenovirus or VEGF-C156Ser had more severity of colitis (Fig.1, histological score $P = 0.008$ or $P = 0.003$), inflammatory edema in the colon submucosa (Fig.2, $P = 0.000$ or $P = 0.033$), and infiltration of inflammatory cells (CD11c+ dendritic cells $P = 0.004$ or $P = 0.002$; MPO+ neutrophils cells in VEGF-C156Ser treated mice, $P = 0.018$) compared with the DSS-treated mice. The significantly increased LVD (Fig.3, adenovirus treated, $P = 0.000$; recombinant protein treated, $P = 0.033$) with enlarged lymphatic vessels and up-regulated level of VEGFR-3mRNA ($P = 0.000$) were found in VEGF-C-treated mice compared with the DSS-treated mice.

CONCLUSIONS: Inflammatory lymphangiogenesis may have pleiotropic effects on the different stage of IBD. Simulation lymphangiogenesis by VEGF-C didn't have the therapeutic effect on the acute colitis in mice, in contrast, aggravates the intestinal inflammation.

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Colonization of Lymphopenic Mice with Dysbiotic Microflora Markedly Increases the Severity and Incidence of Chronic Colitis Following T Cell Transfer

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BACKGROUND: It is well-known that intestinal microbiota are required for the induction of chronic gut inflammation in the $CD4^+CD45RB^{high} \rightarrow$ RAG-$1^{-/-}$ mouse model of chronic colitis. Following our relocation from LSU Health Sciences Center (LSUSHC) to Texas Tech University Health Sciences Center (TTUHSC), we observed a significant reduction in the incidence and severity of disease (30-40%) compared to our previous 15 year history of ~85% at LSUHSC. The objectives of this study were to: a) ascertain whether differences in the gut microbiota may account for the differences in disease incidence and b) determine whether colonization of RAG-$1^{-/-}$ (RAG ko) mice with feces obtained from colitic mice increases the incidence and severity of colitis in the T cell transfer model.

METHODS: DNA from freshly frozen feces was isolated using standard protocols and 16S rRNA sequencing was performed using the Roche 454 platform. For some studies, mice were colonized (via gastric gavage) with 40 mg donor feces 1 week prior to adoptive transfer of naïve ($CD4^+CD45RB^{high}$) T-cells.

RESULTS: Microbiome analysis revealed marked shifts in the relative abundance of several major bacterial communities present in feces obtained from LSUHSC versus TTUHSC mice. For example, the relative abundance of Firmicutes and Bacteroidetes was ~70 and 20%, respectively in healthy TTUHSC-RAG ko mice whereas these same 2 phyla represented 48 and 50%, respectively in healthy LSUHSC-RAG ko animals. Interestingly, the fecal microbiota from TTUHSC-RAG ko mice revealed significantly higher abundances of Proteobacteria, Verrucomicrobia and Tenericutes when compared to the microbiota obtained from healthy LSUHSC-RAG ko mice. Not surprisingly, induction of chronic colitis in LSUHSC-RAG ko mice via adoptive transfer of naïve T cells, produced a marked dysbiosis most notably characterized by an increased abundance of Verrucomicrobia together with corresponding and marked decrease in Bacteroidetes. Adoptive transfer of naive T cells into healthy TTUHSC-RAG ko mice that were first colonized with healthy LSUHSC-RAG ko feces induced mild-to-moderate colitis in ~80% of the mice at 8 weeks post T cell transfer. In addition, we found that T cell transfer into TTUHSC-RAG ko mice that were first colonized with feces from colitic LSUHSC-RAG ko mice induced robust colitis in >90% of these recipients (called LSUHSCc→TTUHSC mice). Furthermore, T cell transfer into TTUHSC-RAG ko mice that had been colonized with feces from colitic LSUHCc→TTUHSC mice induced moderate-to-severe colitis in virtually all (>98%) of these recipients (called TTUHSCc→TTUHSC mice). Importantly, colonization of healthy wild type mice or TTUHSC-RAG ko mice (in the absence of T cell transfer) with colitic feces from TTUHSCc→TTUHSC mice did not induce disease over the 8 week observation period.

CONCLUSIONS: We conclude that colonization of healthy RAG ko mice with dysbiotic microbiota obtained from colitic mice markedly increases the severity and incidence of chronic colitis in the T cell transfer model. Our data also suggest that the increase in severity and incidence of disease is not due to the presence of intestinal pathogens and can only be produced in mice with a dysregulated immune system (supported by grants from the DOD-W81XWH-11-1-0666 and NIH- RO1 DK 091269).

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Ethanol-Induced Enhancement of Disease Severity in DSS Colitis Model

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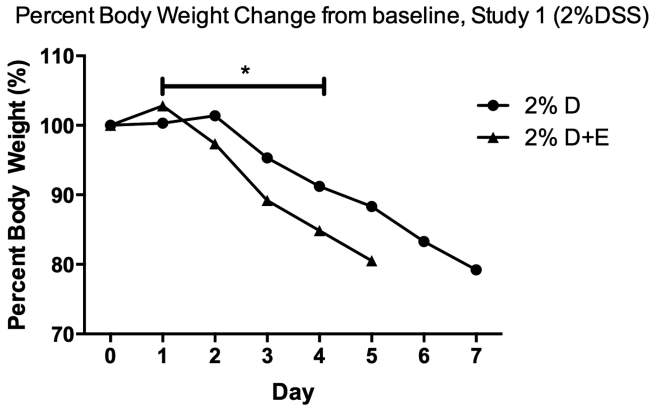

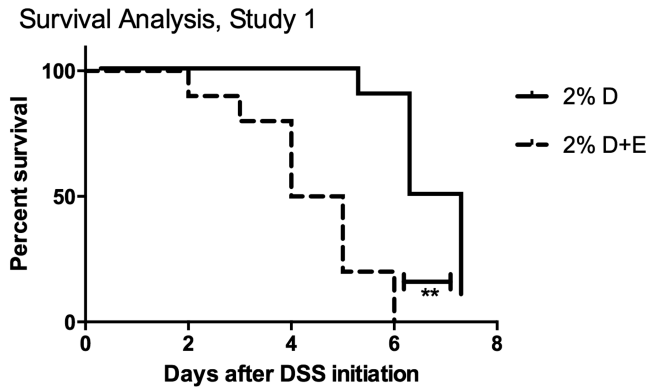

BACKGROUND: Ethanol (EtOH) has been shown to disrupt intestinal epithelial tight junctions and increase intestinal permeability. Few studies have evaluated the role of EtOH consumption and its impact on IBD-related inflammation and disease severity. This Animal Use Committee-approved study aimed to investigate EtOH's role on dextran sulfate sodium (DSS)-induced colitis to determine whether relationship between EtOH consumption and IBD exists, and whether additional epidemiologic research is warranted.

METHODS: Eight week-old C57/Bl6 mice (n = 40) were divided into 4 groups: control diet (C), DSS (D), 5% alcohol (E) and DSS + EtOH (D + E). Both 1% (1%D) and 2% DSS (2%D) were used. Ethanol (5%v/v) was given for 10 days along to group 2% D and 17 days to group 1% D prior to DSS exposure. Control diet consisted of a modified Lieber-DeCarli liquid diet with 40% energy from fat, 43% from carbohydrate and 17% from protein. Diets were made isocaloric by adjusting maltodextrin intake. DSS (w/v) was added to the diets of the DSS treatment groups for a total of 5 days followed by endoscopy and harvest 2 days later. DSS exposure was truncated by 2 days due to excessive mortality in the 2% D + E group. Disease severity was assessed by changes in body weight, rectal bleeding and endoscopic severity score (ESS). To evaluate inflammation, plasma levels of IFN-γ, IL-1β, IL-6, IL-10, IL-17, MCP-1, TNF-α and IL-12 (p40) were measured by cytometric bead array. No 2% D + E animals survived to provide samples for cytokine analysis. Between group comparisons were performed by 2-way ANOVA.

RESULTS: After 5 days of DSS, weight change from baseline were: 1% D (−9%), 1% D + E (−10%), 2% D (−12%), 2% D+E (−19%). After 5 days of DSS, group 2% D + E lost more weight than group 2% D alone ($P = 0.051$). Groups consuming EtOH with DSS consumed less DSS than the DSS only treatment groups: 51% less for 2% D and 10% less for 1% D. Survival rates were as follows: 1% D (33%), 1% D + E (50%), 2% D (10%), 2% D + E (0%). There was no significant survival difference between the 1% treatment groups; however, the survival difference between group 2% D + E and 2% D was significant ($P < 0.01$). Mean ESS were as follows: 1% D (10.7), 1% D + E (8.3), 2% D (13.5). Group 1% D had significantly higher mean stool consistency scores than group 1% D+E ($P < 0.01$). The median onset to rectal bleeding occurred by: day 1 for 2% D + E and day 3 for 1% D, 1% D + E and 2% D. Group 2% D+E developed bleeding significantly earlier than group 2% D ($P < 0.05$). Group 1% D + E demonstrated numerically higher levels of IL-1 β, IL-6, IL-10 and TNF- α versus group 1% D.

CONCLUSIONS: Chronic ethanol consumption enhanced disease severity (rectal bleeding, weight loss and mortality rate) in 2% DSS-induced colitis in mice. Ethanol's